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For: RECOMBINANT PROTEINS AND MOLECULAR COMPLEXES DERIVED FROM  
THESE PROTEINS, ANALOGOUS TO MOLECULES INVOLVED IN IMMUNE  
RESPONSES

\* \* \* \* \*

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Commissioner for Patents  
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Sir:

**SUBMISSION**

Submitted herewith is an English translation of the priority application FR  
99/09862 for the above-identified application.

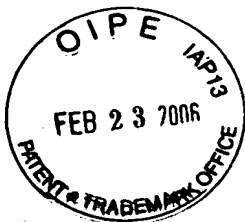
Respectfully submitted,

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5 Recombinant proteins and molecular complexes derived from these proteins, analogous to molecules involved in immune responses.

10 The invention relates to recombinant proteins, and to molecular complexes derived from these proteins, analogous to molecules involved in immune responses.

It also relates to a method for producing such molecules and of such complexes, as well as their uses, in particular for diagnosis and in therapy.

15 It is known that molecules encoded by the Major Histocompatibility Complex (MHC) have a major role in an immune response.

These molecules are made up of two polypeptide chains: the heavy chain, and the light chain.

The molecules of the MHC are expressed on the surface of the presenting cells (dendritic cells, B lymphocytes, macrophages) in the form of molecular complexes with antigenic peptides, which are in turn derived from extracellular or intracellular proteins.

25 Recognition of these peptide/MHC complexes by a specific receptor expressed on the surface of the T lymphocytes is at the origin of any cell-mediated immune response.

The MHC molecules belong to two separate classes: those  
5 of class I, which are recognized by CD8<sup>+</sup> T lymphocytes  
(cytotoxic T cells) and those of class II which are  
recognized by CD4<sup>+</sup> T lymphocytes (helper T cells).

In order to be able to be used as probes for counting  
and measuring the frequency of specific T lymphocytes of a  
10 given antigen, such molecules and complexes have to be  
produced in soluble form. These same soluble molecules and  
complexes can be used for modulating the immune responses.

The possibility of using soluble MHC molecules for  
detecting CD8<sup>+</sup> T lymphocytes was first demonstrated by  
15 Altman et al. in 1996 (1). Since then, many teams have used  
this strategy for counting and characterizing the phenotype  
of CD8<sup>+</sup> T lymphocytes reacting with viral or bacterial  
peptides or peptides derived from tumour antigens. However,  
the application of this strategy for the detection of CD4<sup>+</sup> T  
20 lymphocytes has proved problematic.

In the majority of works published to date, bacterial  
expression systems have been used for producing class I MHC  
molecules. After incubation of these molecules with  
antigenic peptides, the peptide/MHC complexes were purified  
25 and obtained in the form of tetramers after incubation with  
streptavidin. This last stage is made possible by addition,  
to the carboxy-terminal end of the MHC heavy chain, of a  
recognition site for the BirA enzyme, a bacterial enzyme  
that is capable of catalysing the addition of a

biotin molecule. Other teams chose to produce dimers of  
5 class I MHC molecules by using the skeleton of an antibody.  
In this case the MHC heavy chain was bound to the heavy  
chain of an immunoglobulin (abbreviated to Ig) and  $\beta$ -2-  
microglobulin was bound to the light chain. As the Fc  
regions of the heavy chains link together by means of  
10 sulphide bridges, the molecules produced are dimers of MHC  
molecules.

For technical reasons, the preparation of molecular  
probes that bind selectively to the CD4<sup>+</sup> T lymphocytes  
proved much more difficult, probably because of the  
15 intrinsic instability of the class II MHC molecules.

Tetramers of class II molecules bound to an antigenic  
peptide, or dimers of these molecules obtained using the  
skeleton of an antibody, have been produced.

The problem of the stability and affinity of the  
20 receptors of CD4<sup>+</sup> T lymphocytes for their ligand is solved,  
according to the invention, by employing constructs ensuring  
the formation of dimers giving multivalent complexes owing  
to the use of molecules having several binding sites for  
certain regions of the dimers.

25 Such constructs can be envisaged both for class I MHC  
molecules and for those of class II.

Advantageously, the said constructs are sufficiently  
stable for use as

molecular probes, thus opening up a wide field of  
5 application.

These constructs can also be used for obtaining analogues of T cell receptors capable of specifically recognizing such molecules.

The invention therefore aims to supply recombinant  
10 molecules and corresponding recombinant complexes, in which these molecules are bound to antigenic peptides, of great stability and with high affinity for their ligand.

Another aim is their production in host cells with the aid of suitable expression vectors.

15 A further aim of the invention relates to immunological applications of these complexes as molecular probes.

The soluble recombinant proteins according to the invention are constituted, as a minimum, from a dimer, itself formed from  $\alpha$  and  $\beta$  chains of class I or II MHC  
20 molecules.

These dimers are characterized in that they have, at the carboxy terminal end of one or both chains, the whole or part of an Fc region of an immunoglobulin.

"Part of an Fc region" denotes a fragment corresponding  
25 to a natural fragment, or one modified relative to the said natural fragment, by substitution and/or by deletion and/or by mutation, but capable of binding to a protein possessing binding sites for the Fc region, such as protein A or protein G.

30 The term "capable of binding" is illustrated by Example 1C.

The Fc region corresponds more particularly to the whole or part of the CH<sub>2</sub> and/or CH<sub>3</sub> domain. This domain can be modified relative to the natural domain, but must be capable, in accordance with the invention, of binding to a protein of the protein A or G type possessing several binding sites for the Fc region of an Ig.

10 The immunoglobulin having the constant region mentioned above can be an IgG, especially the isotypes IgG1, IgG2a, IgG2b, IgG3, an IgM, an IgA, an IgD or an IgE.

The proteins of the invention are more particularly characterized in that they comprise all or part of the  $\alpha$  or  $\beta$  chains of the MHC molecules.

Advantageously, the  $\alpha$  and  $\beta$  chains constituting the dimer contain leucine zippers, which promotes their pairing.

Such leucine zippers are described for example by Scott et al. (2) or Kalandadze et al. (3).

20 The invention relates in particular to recombinant molecules bound together as several dimers and particularly as tetramers and quite especially as octamers.

The said recombinant molecules are complexed with a natural or artificial protein comprising several binding sites for the constant regions of the immunoglobulins and thus permitting the creation of multimers from dimers. As an example protein A which is commonly isolated from *Staphylococcus aureus*, or protein G from *Streptococcus* (group C), or receptor multimers from the Fc regions obtained by genetic recombination can be mentioned.

The recombinant molecules as defined above, complexed  
5 to antigenic peptides, constitute MHC analogues.

The invention relates to the said complexes,  
characterized in that they have, at the  $-NH_2$  end of the  $\beta$   
chain, an antigenic peptide that is fixed by means of a  
flexible arm. This arm can be of a variable length and makes  
10 it possible to locate the antigenic peptide in the groove  
formed by the dimer or each dimer.

Fixations of this kind are described for example by  
Kozono et al. (4) and (5).

The molecules defined above are advantageously obtained  
15 by the techniques described in textbooks of molecular  
biology for the preparation of recombinant genes and their  
expression in eukaryotic or prokaryotic cells. Reference  
should be made for example to the works of Sambrook et al.  
(6) or of Ausubel et al. (7).

20 The sequences coding for the recombinant fragments  
constituting the molecules defined above are introduced into  
expression vectors. Generally as many expression vectors as  
fragments are used. However, it is also possible, as a  
variant, to use the same vector for several fragments.

25 Plasmids and especially plasmids possessing a selection  
marker will be used advantageously as expression vectors.  
Satisfactory expression results have thus been obtained with  
plasmids that are able to replicate in bacteria and have, as  
selection marker, an antibiotic resistance gene.

30

The promoters will be selected so as to permit  
5 expression of the recombinant gene in the expression system  
used. As an example the promoter recognized by the  
polymerase of the T4 bacteriophage or, when using *Drosophila*  
cells as the expression system, the promoter of the  
metallothionein gene can be mentioned.

10

As eukaryotic expression systems, the recombinant  
baculovirus systems in insect cells, *Drosophila* cells,  
hamster cells (CHO line) and monkey cells (COS line) can be  
mentioned. It is also possible to effect expression in yeast  
15 cells.

Bacteria are widely used, in particular *E. coli*, as  
prokaryotic expression systems.

The recombinant molecules produced are purified on  
immunoaffinity columns, in particular with monoclonal or  
20 polyclonal antibodies specific to the molecules of interest,  
or with supporting materials such as beads, especially  
agarose beads.

Other purification protocols can be envisaged. In  
particular, for example when the molecules to be purified  
25 have 6 consecutive histidine residues, nickel-coated agarose  
beads can be used for purifying the molecules.

The purified molecules obtained are then incubated with  
the proteins possessing the binding sites for the Fc region.

Advantageously, these proteins are labelled for the  
30 purposes of detection, for example with a fluorophore.



When the molecule obtained does not have an antigenic  
5 peptide, and we wish to have available antigenic peptide/MHC  
analogue complexes, it is incubated with the said peptide in  
vitro.

The study of the recombinant molecules according to the  
invention has demonstrated their great stability, and strong  
10 affinity in immunological recognition tests.

The invention thus provides tools that are of  
considerable interest for modulating immunological  
processes.

In particular it relates to the use of antigenic  
15 peptide/class II MHC analogue complexes for counting and/or  
purifying the T lymphocytes that react with a given antigen  
and for characterizing the phenotype of these cells, i.e.  
for determining or identifying the molecules that they  
secrete or that they express on their surface. This  
20 detection is carried out on a sample taken from a patient.  
This can be a blood sample, or a sample taken from secondary  
lymphoid organs, such as the lymph nodes, the spleen, or  
from tumours.

These molecules can be used advantageously for counting  
25 or for purifying these cells from cellular suspensions as  
described above.

Alternatively, they can be used for visualization of  
these cells in cell sections.

It is thus possible to determine the immunological  
30 status of an individual.

This application is of considerable interest for the  
5 development of vaccines against certain pathogens or of  
antitumour vaccines.

It is known that for judging the efficacy of a vaccine,  
the best method is to vaccinate a large number of  
individuals and to monitor what becomes of this population  
10 when it is exposed to the infective agent in natural  
conditions. However, this approach is difficult, notably  
because of the considerable costs involved, and the  
difficulty of finding a sufficient number of volunteers.

The use of complexes according to the invention, as  
15 molecular probes that bind selectively to CD4<sup>+</sup> T lymphocytes  
of given specificity, permits rapid comparison of the  
efficacy of different vaccine preparations and determination  
of the number and the optimum intervals between boosters.

In a preclinical study, individuals are inoculated with  
20 vaccine preparations containing the antigen or antigens,  
then a count is taken of the T cells present in a sample,  
that react with complexes according to the invention. The  
response of the individuals makes it possible to assess the  
reaction to the antigenic peptide.

25 This application can also be employed as predictive  
means as to a patient's condition, by counting and  
determining the phenotype of autoreactive T cells in  
patients at risk.

The invention thus makes it possible to determine the  
30 degree of progression of the disease in patients suffering

from autoimmune diseases or to evaluate the efficacy of  
5 certain treatments or therapeutic interventions.

The invention also relates to the application of the  
said multivalent complexes defined above in the diagnosis  
and development of treatments for autoimmune diseases.

A certain number of autoimmune diseases are due to the  
10 mobilization of autoreactive T lymphocytes that cause the  
destruction of the organism's tissues. In some cases, for  
example in diabetics, the disease is diagnosed late, when  
the tissues are already destroyed. To prevent the  
destruction of tissues, and block the development of the  
15 disease, it is essential to make an early diagnosis. The  
possibility of counting, by means of the invention, the  
autoreactive T lymphocytes in the blood of patients at risk  
constitutes a considerable advance.

Taking into account that the autoreactive T lymphocytes  
20 play a decisive role in the development of autoimmune  
diseases, very many therapeutic strategies aim to eliminate  
these lymphocytes, or prevent them exerting their  
pathogenicity, it can be seen that there is a great  
advantage in being able to count, by means of the invention,  
25 the autoreactive T lymphocytes in the blood of treated  
patients, to compare the efficacy of different treatments,  
and to adapt the treatment according to the patient's  
response.

According to another aspect, the invention relates to  
30 the use of the complexes for enrichment in a given type of T  
cells.

This application makes it possible to have available  
5 large quantities of specific T cells of a given antigen in  
vitro for purposes of cellular therapy. The patients can in  
fact be reinoculated with these cells for prevention or  
cure. Once again it is possible to count and determine,  
prior to inoculation, the phenotype of the complexed T  
10 cells.

The invention further relates to the application of  
multivalent recombinant molecules as T-cell-stimulating  
agents.

An individual can be inoculated with these molecules in  
15 order to stimulate the expansion and/or the activation of  
specific T cells of a given antigen in the absence of any  
other cell, in particular of presenting cells.

This use is therefore of interest for stimulating  
inadequate immune responses, for example with respect to  
20 MHC/tumour antigen complexes.

In the case of infectious diseases, the recombinant  
molecules are inoculated *in vivo*, if necessary after a  
previous stage of propagation *ex vivo*.

Other characteristics and advantages of the invention  
25 are given, purely for illustration, in the examples given  
below and refer to Figures 1 to 5, which show, respectively:

- Figure 1 shows the sequence of the cDNA insert of the  
 $\alpha$  chain of the MHC,

30

- Figure 2 shows the plasmid construct containing the  
cDNA insert of Figure 1,

- Figure 3 shows the sequence of the cDNA insert of the  $\beta$  chain of the MHC,

5

- Figure 4 shows the plasmid construct containing the cDNA insert of Figure 3,

- Figure 5 shows the detailed plasmid construct of Figure 4, and

10

- Figure 6 shows a peptide/class II MHC octamer according to the invention.

15

#### Example 1: Production of peptide/class II MHC complexes

##### 1. Construction of the recombinant plasmids

. cDNA construct coding for the IA $\alpha^d$ /Fc recombinant protein (clone 461) and insertion in a plasmid

This construct is illustrated by Figure 1 which gives the cDNA sequence, from position 420 to 1940, and that of the coded peptide (437-1921) (SEQ ID No. 1).

The cDNA comprises, linked together successively, the fragments coding for the signal peptide of IA $\alpha^d$ , IA $\alpha^d$ , a linker, an acidic leucine zipper, a linker, a hinge region, the CH<sub>2</sub> region, then the CH<sub>3</sub> region of Fc.

25

This construct is inserted in the plasmid shown in Figure 2 and positioned for the control of a CuSO<sub>4</sub>-inducible metallothionein promoter.

30

. cDNA construct coding for the recombinant LACK protein /I-A  $\beta^d$ /leucine zipper (clone 268) and insertion in a plasmid

This construct is shown in Figure 3, which gives the cDNA sequence, from position 420 to 1370, and that of the coded peptide (440-1359) (SEQ ID No. 2).

The cDNA comprises successively the fragments, linked together, : coding for a leader sequence,  $\beta 1$ , a LACK peptide (158-73), a linker, a thrombin site, a linker,  $IA\beta^d$  ( $\beta 1$ )  $IA\beta^d$  ( $\beta 2$ ), a linker, a basic leucine zipper, a marker with histidine units.

This construct is inserted in the plasmid shown in Figure 4, and shown in detail in Figure 5.

## 2. Transfection of the plasmids in Drosophila cells

### 3. Selection of stable transmitters

Stages 2 and 3 are carried out following the procedure according to (6).

## 4. Production and purification of the complexes

### A) Production

The transfected Drosophila cells are cultured in 3-litre bottles, at 24°C, in an SFM Drosophila medium (GIBCO-BRL), supplemented with 1% of FCS (fetal calf serum).

When the cell density reaches  $5 \times 10^6$  cells/ml, the production of LACK/IAd molecules is induced by adding  $CuSO_4$  to a final concentration of 1 mM, then the medium is incubated for 5 to 6 days.

The supernatants are combined, and the cell debris is eliminated by centrifugation (20 min, 10K, 4°C). The supernatants are then transferred to tubes and centrifuged again.

The supernatants are concentrated 8 to 10-fold using a Prepscale<sup>R</sup> concentrator (Millipore, Inc.). Freezing is effected at -70°C until 500 ml of concentrated supernatants is obtained.

#### B) Purification

The supernatants are thawed at 37°C. Centrifugation is carried out for 15 minutes at 10K. The supernatants are then transferred to new tubes and are centrifuged again for 15 minutes at 10K.

They are then charged on an MK-D6 immunoaffinity column (bed volume 5 ml), equilibrated beforehand in a buffer A of 20 mM of sodium phosphate pH 7.0. The rate of elution is 10 to 20 ml/h.

The column is washed with 30 ml of buffer A (6 times the volume of the bed) at 0.5 ml/min.

For elution 15 ml of CAPS 50 mM pH 11.5 is used, operating by gravity.

15 fractions, each of 1 ml, are collected.

Each fraction is neutralized with 300 µl of sodium phosphate (200 mM, pH 6.2). Protease inhibitors (Complete<sup>R</sup>, Roche Diagnostics) are added to each sample immediately.

The column is neutralized with buffer A.

To prevent aggregation of the peptide/MHC molecules, ion exchange chromatography is carried out immediately after elution.

The protein concentration in each fraction is determined by electrophoresis in denaturing gel.

5 The positive fractions are combined and loaded on an ion exchange column (Mono Q) (Pharmacia Biotech).

A buffer B is used: Tris-HCl 20 mM, pH 8.0, and a buffer C: Tris-HCl 20 mM pH 8.0 + 1 M NaCl.

Operation is effected with the following gradients:

0-5 min: 0% C; 5-20 min: 0-50% C; 20-21 min: 50-100% C;  
10 21-25 min: 100% C; 25-26 min: 100% C; 26-30 min: 0% C.

The LACK/IA<sup>d</sup> molecules generally elute to 30-36% in buffer C. The fractions corresponding to the elution peak are collected and the protein concentration is determined by electrophoresis in denaturing gel.

15 The positive fractions are combined and are dialyzed at 4°C against 2 l of PBS, pH 7.4.

The dialysis buffer is changed twice in 24 h. The protein concentration is determined by the BCA test (Biorad). The samples are frozen at -70°C in small fractions  
20 (8 µg). The yields are of the order of 0.5 mg/l of cellular supernatant.

#### C. Production of multivalent complexes (Figure 6)

25 A solution of protein A is prepared, coupled to a fluorophore consisting of Alexa 488<sup>R</sup> (molecular probes # P-11047) at a concentration of 0.5 mg/ml in PBS 1 X, pH 7.4. (Protein A from Sigma)

100 µl aliquots are prepared and frozen at -20°C.

30



A peptide/MHC molecule aliquot (8  $\mu$ g) is thawed and 1.1  
5  $\mu$ l of protein A coupled to the Alexa fluorophore is added.  
The mixture is incubated at room temperature for 30 min,  
then a PBS/BSA (bovine serum albumin) 0.1% mixture is added  
to give a final volume of 50  $\mu$ l. 1  $\mu$ l of mouse serum is  
added, and the product is used directly as staining reagent.

10

#### D. Flow cytofluorimetry

T cells from mouse lymph nodes are purified.  $10^6$  cells  
15 are transferred to a tube and the staining reagent is added.  
Two hours later, the cells are washed in isotonic buffer and  
are analyzed by flow cytofluorimetry. The frequency of cells  
reacting with the staining reagent is determined by this  
method.

20

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CLAIMS

5           1. Soluble recombinant proteins, constituted as a  
minimum from a dimer that is itself formed from  $\alpha$  and  $\beta$   
chains of class I or II MHC molecules, characterized in that  
they comprise at the carboxy-terminal end of one or both  
chains, the whole or part of an Fc region of an  
10 immunoglobulin.

          2. Soluble recombinant proteins according to claim 1,  
characterized in that they comprise all or part of the  $\alpha$  and  
 $\beta$  chains of MHC molecules.  
15

          3. Soluble recombinant proteins according to claim 1 or  
2, characterized in that they comprise all or part of the  
CH2 and/or CH3 area of the Fc region.

20           4. Soluble recombinant proteins according to any one of  
claims 1 to 3, characterized in that the chains which  
constitute the dimer comprise leucine zippers.

          5. Soluble recombinant proteins according to any one of  
25 claims 1 to 4, characterized in that they are combined in  
several dimers and in particular in tetramers or in  
octamers.

          6. Soluble recombinant proteins according to any one of  
30 claims 1 to 5, characterized in that they are complexed with  
natural or artificial proteins, comprising several binding  
sites for

the constant regions of immunoglobins such as protein A,  
protein G or receptor multimers of the Fc regions obtained  
5 by genetic recombination.

7. Soluble recombinant proteins according to any one of  
claims 1 to 6, characterized in that they are bound  
covalently or noncovalently to an antigenic peptide.  
10

8. Soluble recombinant proteins according to claim 7,  
characterized in that the antigenic peptide is fixed to the  
amino-terminal end of the  $\beta$  chain by means of a flexible  
arm.  
15

9. Nucleotide sequences possessing a reading frame  
corresponding to all or part of a molecule according to any  
one of claims 1 to 8.

10. Expression vectors, in particular plasmids,  
characterized in that they have a sequence according to  
claim 9.  
20

11. Prokaryotic or eukaryotic cells carrying at least  
one vector according to claim 10.  
25

12. Use of the molecules according to claim 7 or 8, for  
counting and/or purifying the T lymphocytes that react with  
a given antigen and for characterizing the phenotype of  
these cells.  
30

13. Use according to claim 12, as immunostimulating  
proteins, in particular for the development of vaccines.

14. Use according to claim 12, as a means of predicting  
a patient's condition, for counting and determining the  
5 phenotype of autoreactive T cells in patients at risk, or  
for therapeutic purposes.

15. Use of the molecules according to claim 7 or 8, for  
the purification and/or enrichment of specific T lymphocytes  
10 of a given antigen, either from cell cultures, or from  
samples taken from a patient.

16. Use according to claim 15, characterized in that  
the populations of T lymphocytes enriched with a given type  
15 of T cells, are used for the purposes of cellular therapy.

The recombinant molecules as defined above, complexed to antigenic peptides, constitute MHC analogues. These are soluble recombinant proteins, characterized in that they are bound covalently or non-covalently to an antigenic peptide. The invention relates to the said complexes, characterized in that they have, at the  $-NH_2$  end of the  $\beta$  chain, an antigenic peptide that is fixed by means of a flexible arm. This arm can be of a variable length and makes it possible to locate the antigenic peptide in the groove formed by the dimer or each dimer. Fixations of this kind are described for example by Kozono et al. (4) and (5).

The molecules defined above are advantageously obtained by the techniques described in textbooks of molecular biology for the preparation of recombinant genes and their expression in eukaryotic or prokaryotic cells. Reference should be made for example to the works of Sambrook et al. (6) or of Ausubel et al. (7).

The nucleotide sequences of the invention possess a reading frame corresponding to the whole or part of a protein as defined above.

The sequences coding for the recombinant fragments constituting the molecules defined above are introduced into expression vectors. Generally as many expression vectors as fragments are used. However, it is also possible, as a variant, to use the same vector for several fragments. Plasmids and especially plasmids possessing a selection marker will be used advantageously as expression vectors. Satisfactory expression results have thus been obtained with plasmids that are able to replicate in bacteria and have, as selection marker, an antibiotic resistance gene.

the constant regions of immunoglobins such as protein A,  
5 protein G or receptor multimers of the Fc regions obtained  
by genetic recombination.

7. Soluble recombinant proteins according to any one of  
claims 1 to 6, characterized in that they are bound  
10 covalently or noncovalently to an antigenic peptide.

8. Soluble recombinant proteins according to claim 7,  
characterized in that the antigenic peptide is fixed to the  
amino-terminal end of the  $\beta$  chain by means of a flexible  
15 arm.

9. Nucleotide sequences possessing a reading frame  
corresponding to all or part of a protein according to any  
one of claims 1 to 8.

20 10. Expression vectors, in particular plasmids,  
characterized in that they have a sequence according to  
claim 9.

25 11. Prokaryotic or eukaryotic cells carrying at least  
one vector according to claim 10.

12. Use of the proteins according to claim 7 or 8, for  
counting and/or purifying the T lymphocytes that react with  
30 a given antigen and for characterizing the phenotype of  
these cells.

13. Use according to claim 12, as immunostimulating  
proteins, in particular for the development of vaccines.

14. Use according to claim 12, as a means of predicting  
a patient's condition, for counting and determining the  
5 phenotype of autoreactive T cells in patients at risk, or  
for therapeutic purposes.

15. Use of the proteins according to claim 7 or 8, for  
the purification and/or enrichment of specific T lymphocytes  
10 of a given antigen, either from cell cultures, or from  
samples taken from a patient.

16. Use according to claim 15, characterized in that  
the populations of T lymphocytes enriched with a given type  
15 of T cells, are used for the purposes of cellular therapy.



## CLAIMS

1. Soluble recombinant proteins, constituted as a  
5 minimum from a dimer that is itself formed from  $\alpha$  and  $\beta$   
chains of class I or II MHC molecules, characterized in that  
they comprise at the carboxy-terminal end of one or both  
chains, the whole or part of an Fc region of an  
immunoglobulin, in particular, all or part of the CH<sub>2</sub> and/or  
10 CH<sub>3</sub> domain of the Fc region, the chains which constitute the  
dimer containing leucine zippers, and being bound together  
in several dimers and particularly in tetramers or in  
octamers and complexed with natural or artificial proteins,  
comprising several binding sites for the constant regions of  
15 the immunoglobulins such as protein A, protein G or receptor  
multimers from the Fc regions obtained by genetic  
recombination.

2. Soluble recombinant proteins according to claim 1,  
20 characterized in that they are bound covalently or non-  
covalently to an antigenic peptide.

3. Soluble recombinant proteins according to claim 2,  
characterized in that the antigenic peptide is fixed to the  
25 amino-terminal end of the  $\beta$  chain by means of a flexible  
arm.

4. Nucleotide sequences possessing a reading frame  
corresponding to all or part of a protein according to any  
30 one of claims 1 to 3.

5. Expression vectors, in particular plasmids,  
characterized in that they have a sequence according to  
claim 4.

## CORRECTED SHEET

6. Prokaryotic or eukaryotic cells carrying at least one vector according to claim 5.

5

7. Use of the proteins according to claim 2 or 3, for counting and/or purifying the T lymphocytes that react with a given antigen and for characterizing the phenotype of these cells.

10

8. Use according to claim 7, as immunostimulating proteins, in particular for the development of vaccines.

9. Use according to claim 7, as a means of predicting a patient's condition, for counting and determining the phenotype of autoreactive T cells in patients at risk, or for therapeutic purposes.

10. Use of the proteins according to claim 2 or 3, for the purification and/or enrichment of specific T lymphocytes of a given antigen, either from cell cultures, or from samples taken from a patient.

11. Use according to claim 10, characterized in that the populations of T lymphocytes enriched with a given type of T cells, are used for the purposes of cellular therapy.